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Near-Infrared Transmission and Reflectance Spectroscopy for the Determination of Dietary Fiber in Barley Cultivars

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ABSTRACT

Dietary fiber is an important quality parameter of barley (*Hordeum vulgare* L.) but is extremely laborious to measure. Near-infrared (NIR) transmission and reflectance spectroscopy were investigated as rapid screening tools to evaluate the total dietary fiber content of barley cultivars. The Foss Grainspec Rice Analyzer and NIR Systems 6500 spectrometer were used to obtain transmission and reflectance spectra, respectively, of polished grains and ground barley. Total dietary fiber was determined for each cultivar by AOAC Method 991.43. Modified PLS models developed for predicting total dietary fiber, using transmission spectra (850–1048 nm) of polished grains, had a standard error of cross validation (SECV) of 10.4 (range 58–197) g kg^{-1} and R^2 of 0.82 indicating sufficient accuracy for selecting or rejecting high dietary fiber cultivars. NIR reflectance spectroscopy (1104–2494 nm) of ground barley samples resulted in a model with SECV of 5.2 (range 58–197) g kg^{-1} and R^2 0.96, indicating a high degree of precision in the prediction of total dietary fiber. The increased accuracy of the reflectance model may be due in part to more information available in the wavelength region used. The precision, low cost per sample and speed of measurement of the technique allow making dietary fiber selection decisions for large numbers of progeny in barley breeding programs.

DIETARY FIBER is an important quality parameter for barley cultivars. High fiber content is undesirable in cultivars used for brewing and animal feed and desirable in barley cultivars used for food production. The primary use of barley is for production of malt or germinated barley used as a source of fermentable sugars for the production of beer and whiskey. Other uses include feed for animals in northern climates where corn (*Zea mays* L.) cannot be grown, as a staple for human food, and for the production of waxy starch used in food processing (Andersson, 1999). Fiber extracted from barley has potential in functional and fortified foods such as snack bars and beverages. Additional food uses of barley in Japan include barley tea, shochu, miso, and as a rice extender.

Although to date the production of barley for human food has been minor, the fact that some cultivars contain considerable quantities of fiber, particularly soluble fiber, present primarily in the form of β-glucan (Fincher, 1975), makes barley of greater interest as a source of dietary fiber. High dietary fiber intake, particularly of viscous fibers such as β-glucan, is associated with lowering of blood cholesterol levels and normalizing blood glucose and insulin, making foods containing these fibers an important part of dietary strategies to minimize cardiovascular disease and type-2 diabetes (American Dietetic Association, 2002).

In contrast to its benefits in food, fiber is detrimental during malting and brewing. In brewing, a high soluble fiber content, particularly of β-glucan, causes blocking of filters and reduced recovery of fermentable sugars (see review by Bamforth, 1985). Furthermore, high β-glucan content can lead to colloidal instability and haze formation in the final product. The major components of barley are protein, fiber, and starch; however, there appears to be a wide range in genetic variation of these components (Oscarsson et al., 1996; Bhatty, 1999; Andersson, 1999). Globally, covered or hulled barley is the most widely produced and is the traditional barley used for malting and brewing. Alternatively, naked or hullless barley is most often used for food since it does not require processing to remove the hull, and thus nutrients and bioactive components are retained.

The fiber content of plant material is measured as total dietary fiber (AOAC, 1992), which, by definition, includes plant nonstarch polysaccharides, oligosaccharides, fructans, resistant starch, and lignin (AACC, 2001). This is in contrast to crude fiber, acid detergent fiber, and neutral detergent fiber methods, which do not measure all of the individual components (Dreher, 1987). Conventional dietary fiber analysis is extremely time consuming and labor intensive. The procedures used for total, soluble, and insoluble dietary fiber are enzymatic–gravimetric methods or enzymatic chromatographic methods that take 2 to 3 d to perform and are labor intensive (e.g., AOAC, 1992, Englyst et al., 1982). It would be beneficial in breeding programs to have a rapid method that would allow evaluating the fiber content of hundreds of samples per day.

NIR spectroscopy is an analytical technique that is rapid, requires very little labor once a calibration is obtained, and does not require chemicals or, therefore, create chemical waste. The technique has extensive application for the analysis of constituents of agricultural crops, feeds, and foods (Williams and Norris, 1987, 2001; Marten et al., 1989; Osborne et al., 1993; Roberts et al., 2004). Previous work has demonstrated the potential of NIR spectroscopy for the rapid evaluation of neutral detergent fiber, total dietary fiber, and the components of dietary fiber in wheat bran mixtures, oat bran, and diverse cereal products (Baker , 1983; Horváth et al.,

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Abbreviations: NIR, near-infrared; PLS, partial least squares; R^2, coefficient of determination; r^2, multiple coefficient of determination; SD, standard deviation; SEC, standard error of calibration; SECV, standard error of cross validation; TDF, total dietary fiber.

1984; Williams et al., 1991; Kays et al., 1996, 1998; Archibald and Kays, 2000; Kays and Barton, 2002). NIR spectroscopy has also been investigated as a tool for the evaluation of barley protein and moisture content and for the quality evaluation of malt (Henry, 1985; Angelino, 1996; Lu et al., 2000).

NIR spectroscopy, while much simpler and more rapid than traditional analytical methods, typically requires grinding a sample to a fine particle size to give a relatively smooth and homogeneous surface for reflection and increased precision. Near-infrared transmission spectroscopy does not require sample grinding as the radiation passes through the sample and is measured on the side opposite to the incident radiation. Currently, near infrared transmission spectroscopy of whole grains at the grain elevator is used widely in the USA, Canada, Australia, and Europe for the evaluation of protein and moisture content of wheat. For screening in barley breeding programs, it would be advantageous to be able to evaluate total dietary fiber in intact or polished barley grains rapidly and without the need for grinding. However, little is known about the potential of NIR transmission or reflectance spectroscopy for the evaluation of total dietary fiber in barley. An earlier study published in the Japanese language (Shimizu et al., 2002) described the development of a transmission calibration for total dietary fiber using a limited number of barley samples. The current study expands the calibration and investigates the potential of both NIR transmission and reflectance spectroscopy for the screening of breeding lines of hulled and hull-less barley for total dietary fiber content (TDF).

MATERIALS AND METHODS

Samples and Sample Preparation

Hulled (n = 24) and hull-less (n = 32) samples of barley were obtained from the National Agricultural Research Center, Western Region, Zentsuji City, Kagawa Prefecture, Japan, and spanned harvests from 1995 through 1999. Grains were polished with a Satake Grain Testing Mill TM-5 (Satake Co. Ltd., Hiroshima, Japan) to 40% weight loss for hull-less barley and 45% weight loss for hulled barley. Subsamples of polished barley were ground to <500 μm in a Retche Ultracentifuge Mill (Germany) before reflectance measurements and TDF analysis.

Spectroscopic Analysis

Polished barley samples were scanned in triplicate with a Grainspec Rice Analyzer (850–1048 nm) (Foss-Tecator, Hoganos, Sweden) to obtain transmission spectra. Near-infrared reflectance spectra of ground barley samples were obtained in triplicate with an NIRSytems 6500 monochromator (400–2498 nm) (Foss-NorthAmerica, Silver Spring, MD) using a spinning cup sampling device.

Reference Analysis of Total Dietary Fiber

Total dietary fiber was determined by the AOAC enzymatic gravimetric method (AOAC Method 991.43; AOAC, 1992), residual protein in the fiber extract by combustion analysis (AOAC Method 992.23, AOAC, 1995), and moisture content of the samples by a forced air oven method (AOAC Method 945.15, AOAC, 1990). Total dietary fiber was expressed on a dry weight basis.

Calibration Development

Multivariate analysis was performed by a commercial analysis program (NIRS 3 version 4.01, Infrasoft International Inc., Port Matilda, PA) to relate the spectral data, obtained from each of the two instruments, to the dietary fiber values. Data were centered using PLS1 and spectral outliers identified. One sample (a hull-less barley cultivar, 'Hadakamugi Chuukan Botino') was identified as a spectral outlier with each instrument (Mahalanobis distance 5.7 and 8.6) and removed from the data set. Separate calibrations, for each instrument, were developed for the prediction of total dietary fiber by modified PLS as the regression method. Spectra were preprocessed with multiplicative scatter correction, to partially correct for baseline differences and, in the case of ground barley, second derivative processing. The optimum number of factors in the model was determined by cross validation. Performance of the PLS models is reported as (i) the calibration standard error and coefficient of determination and (ii) the standard error of cross validation, multiple coefficient of determination, bias, and slope of the linear regression of the TDF values determined by the AOAC method versus the TDF predicted by the NIR calibration.

RESULTS AND DISCUSSION

The overall range of total dietary in the cultivars tested was from 57.6 to 197.1 g kg^{-1} on a dry weight basis (Table 1). The range of total dietary fiber was wide for both the hulled and hull-less cultivars with no significant difference in the overall means between hulled and hull-less cultivars used in the study (Students t test, $p > 0.05$); thus, the genetic diversity in dietary fiber content is high for both hulled and hull-less barley. The standard error of the laboratory determinations (pooled standard error of repeatability) of the enzymatic–gravimetric method for total dietary fiber determination across barley cultivars was 3.7 g kg^{-1} (ASTM, 1995).

The NIR spectra obtained were typical of barley samples and cereal grains in general (data not shown; Williams and Norris, 1987). For polished barley, using the Grainspec Rice Analyzer (850–1048 nm) a NIR calibration for the prediction of total dietary fiber was developed using a multiplicative scatter correction, to reduce particle size effects, and modified PLS regression. The number of factors in the model, determined by cross validation, was seven (Fig. 1), and the SECV and multiple coefficient of determination were 10.4 g TDF kg^{-1} and 0.82, respectively (Table 2, Fig. 2A). According to the guidelines for NIR model development (ASTM, 1995), the number of factors that may be used in a multivariate model is limited by the number of samples used in development of the calibration. "If a model is

Table 1. Total dietary fiber composition (g kg^{-1}) of barley cultivars.

Cultivars	n	TDF†	Range
All	55	94.7 ± 25.9	57.6–197.1
Hulled	24	82.4 ± 18.8	57.6–147.0
Hull-less	31	103.9 ± 24.8	84.4–197.1

† Mean ± SD.

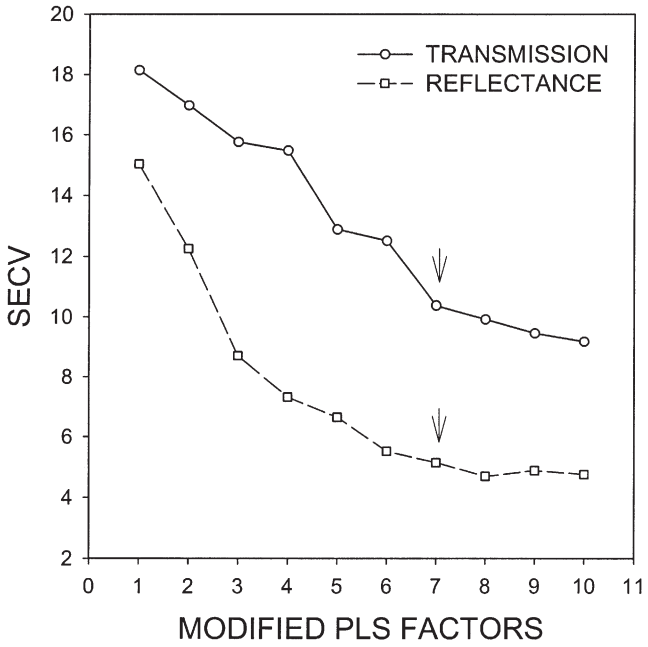

Fig. 1. Number of modified PLS factors versus standard error of cross validation (SECV) for NIR transmission and reflectance models to predict total dietary fiber (g kg^{-1}) in polished and ground barley, respectively. Arrows denote the number of factors used for the corresponding models.

developed with k (>3) variables, then the calibration set should contain a minimum of 6k spectra after elimination of outliers" (ASTM, 1995). For seven factors, as used in the current models, a minimum of 42 samples is required; thus, the number of factors is reasonable given the size of the calibration set.

A NIR calibration for prediction of total dietary fiber was developed for ground barley samples and scanned with the NIRSystems 6500 in reflectance mode. Using the wavelength range of 1104 to 2494 nm and spectra processed with a multiplicative scatter correction and second derivative, a modified PLS regression calibration was obtained with seven PLS factors (Fig. 1). The SECV and multiple coefficient of determination for the model were 5.2 g TDF kg^{-1} and 0.96, respectively (Table 2; Fig. 2B). PLS loadings show the regression coefficients of each wavelength to a constituent and can indicate which wavelengths are important in development of a model. Examination of the first PLS loading for the model showed high variation in absorption in regions of the spectrum associated with O–H groups in water at 1416 and 1908 nm, C–H groups in the carbohydrate

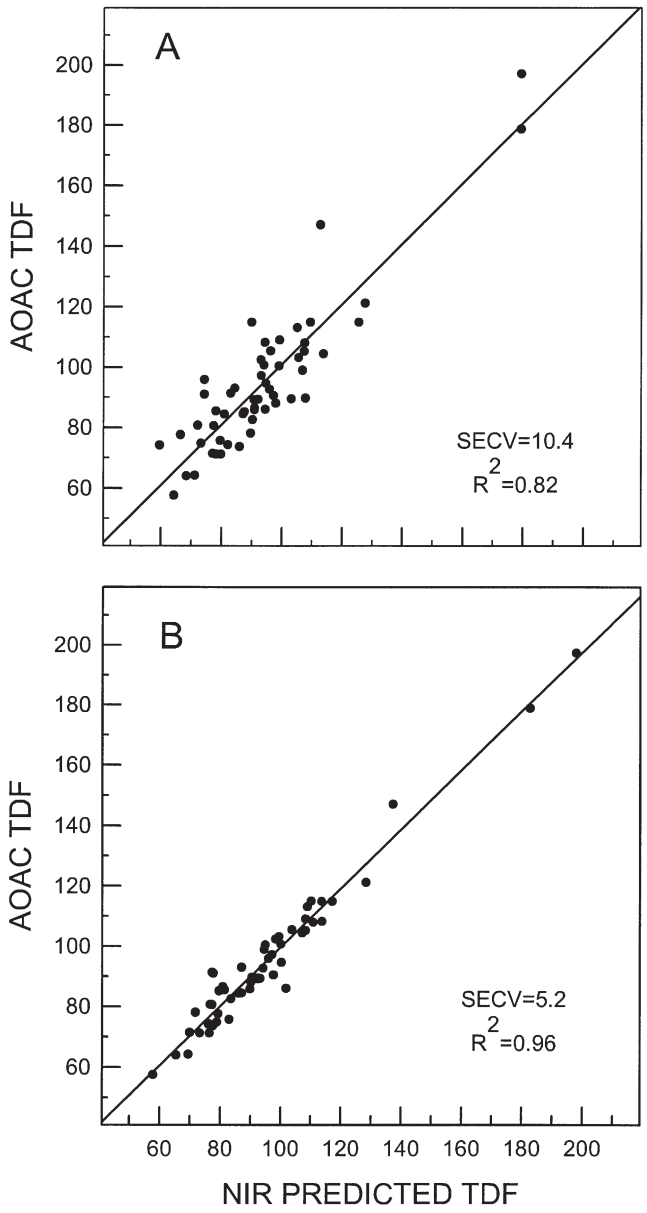

Fig. 2. AOAC determined versus NIR predicted total dietary fiber (g kg^{-1}) in barley cultivars using NIR transmission (A) and reflectance spectroscopy (B).

band at 2268 nm, in the aliphatic C–H band at 2310, and protein band at 2058, indicating that these regions were important in model development. This result is similar to the loadings for the calibration for total di-

Table 2. Calibration and cross validation statistics for prediction of dietary fiber (g kg^{-1}) in barley cultivars by NIR transmission and reflectance spectroscopy.†

				Calibration		Cross validation					
Method	n	Range	PLS Factors	SEC	R^2	Mean	SD	SECV	r^2	Bias	Slope
AOAC	55	57.6–197.1				94.7	24.5				
NIR Transmission Grainspec Rice Analyzer*	55		7	8.4	0.88	93.8	22.3	10.4	0.82	0.90	1.00
NIR reflectance NIRSystems 6500**	55		7	3.6	0.98	94.9	24.6	5.2	0.96	−0.20	0.98

† NIR = near-infrared; n = number of samples; PLS = partial least squares; SEC = standard error of calibration; R^2 = coefficient of determination; SD = standard deviation; SECV = standard error of cross validation; r^2 = multiple coefficient of determination.

etary fiber in ground, processed cereal products (Kays et al., 1996).

The model for TDF, developed with the ground samples and reflectance spectra, was more precise than that developed with the intact samples and transmission spectra. Transmission measurements have very little scatter and typically have higher precision than reflectance measurements. However, when the standard errors are higher for transmittance measurements, it is usually because less information is available because of a limited wavelength range. The PLS loadings most highly correlated to dietary fiber prediction in ground samples in previous studies (Kays et al., 1996, 1998), and the current study have high variation in the 2200- to 2300-nm range, which is not measured by the Grainspec Analyzer used for transmission measurements in this study. In addition, there may be less penetration of light into intact rather than ground samples. In fact, reduced precision was also observed in a study to predict TDF by NIR in intact versus ground processed cereal products using NIR reflectance spectra (Kays et al., 1996; Archibald and Kays, 2000). However, the advantages of scanning cultivars and products without the necessity of grinding are highly significant when large numbers of samples are evaluated.

A previous calibration for total dietary fiber in ground, low fat, and low sugar cereal products ($n = 90$) used nine PLS factors and had a SECV and multiple coefficient of determination of 16 (range $<$ 10–520) g TDF kg^{-1} and 0.99, respectively. The standard error of performance and coefficient of determination, when predicting independent validation samples, were 15 (range $<$ 10–440) g TDF kg^{-1} and 0.99, respectively (Kays et al., 1996). The model was accurate enough for quality control and for monitoring cereal product nutrition label information. Although the range error ratio (RER, ratio of the reference method measured range of TDF to the SECV of the NIR model) is smaller for prediction of TDF in polished barley, both the polished barley model (RER = 13.4) and the model developed with ground barley (RER = 26.8) appear to be sufficiently accurate for screening samples, and the latter is sufficiently accurate for quality control applications (AACC, 2000; Williams, 2001). Additional tests with a calibration data set containing more samples in the upper part of the dietary fiber range could provide improvements in the accuracy of the model, as could the use of a transmission instrument with wider potential wavelength range.

One advantage of NIR technology is the ability to predict numerous parameters simultaneously with one spectrum. Thus, NIR spectroscopy has considerable potential for the selection of cultivars for multiple components, in addition to dietary fiber, simultaneously in large numbers of barley progeny.

CONCLUSIONS

Near infrared reflectance spectroscopy of ground barley appears to be an accurate method for the determination of total dietary fiber content. Near infrared transmission spectroscopy of polished barley grains is less accurate but appears to provide sufficient accuracy for selecting or rejecting high total dietary fiber cultivars or progeny and requires less sample preparation time. The reduced accuracy of the transmission method may be due, in part, to lower penetration of light into the intact barley and a narrower wavelength range with less information available for dietary fiber analysis in the wavelength region used.

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